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AD-A227 553

REPORT DATE	3 REPORT TYPE AND DATES COVERED
1990	Reprint

(see title on reprint)

5 FUNDING NUMBERS
Program Element No. NWED QAXM
Work Unit No. 00132

6 AUTHOR(S)

Patchen et al.

7 PERFORMING ORGANIZATION NAME(S) AND ADDRESS(ES)	8 PERFORMING ORGANIZATION REPORT NUMBER
Armed Forces Radiobiology Research Institute Defense Nuclear Agency Bethesda, MD 20889-5145	SR90-19

9 SPONSORING/MONITORING AGENCY NAME(S) AND ADDRESS(ES)	10 SPONSORING/MONITORING AGENCY REPORT NUMBER
Defense Nuclear Agency Washington, DC 20305	

11 SUPPLEMENTARY NOTES

12a. DISTRIBUTION/AVAILABILITY STATEMENT	12b. DISTRIBUTION CODE
Approved for public release; distribution unlimited.	

13 ABSTRACT (Maximum 200 words)

DTIC
ELECTE
OCT 12 1990
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14 SUBJECT TERMS			15 NUMBER OF PAGES
			16
			16 PRICE CODE

17 SECURITY CLASSIFICATION OF REPORT	18 SECURITY CLASSIFICATION OF THIS PAGE	19 SECURITY CLASSIFICATION OF ABSTRACT	20 LIMITATION OF ABSTRACT
UNCLASSIFIED	UNCLASSIFIED		

Original Paper

Therapeutic Administrati)n of Recombinant Human Granulocyte Colony-Stimulating Factor Accelerates Hemopoietic Regeneration and Enhances Survival in a Murine Model of Radiation-Induced Myelosuppression

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Abstract. The primary cause of death after radiation exposure is infection resulting from myelosuppression. Because granulocytes play a critical role in host defense against infection and because granulocyte proliferation and differentiation are enhanced by granulocyte colony-stimulating factor (G-CSF), this agent was evaluated for the ability to accelerate hemopoietic regeneration and to enhance survival in irradiated mice. C3H/HeN mice were irradiated and G-CSF (2.5 μg/day, s.c.) or saline was administered on days 3-12, 1-12 or 0-12 post-irradiation. Bone marrow, splenic and peripheral blood cellularity, and bone marrow and splenic granulocyte-macrophage progenitor cell recoveries were evaluated in mice exposed to 6.5 Gy. Mice exposed to 8 Gy were evaluated for multipotent hemopoietic stem cell recovery (using endogenous spleen colony-forming units) and enhanced survival. Results demonstrated that therapeutic G-CSF 1) accelerates hemopoietic regeneration after radiation-induced myelosuppression, 2) enhances survival after potentially lethal irradiation and 3) is most effective when initiated 1 h following exposure.

Introduction

Colony-stimulating factors (CSFs) are glycoprotein growth factors capable of controlling the survival, proliferation and differentiation of hemopoietic progenitor cells [1, 2]. At least four different CSFs that affect granulocyte (G-CSF), macrophage (M-CSF/CSF-1), granulocyte-macrophage (GM-CSF) and multipotential (Multi-CSF/IL-3) progenitor cells have been identified. Although the

action of some CSFs appears to be species-specific, several human CSFs cross-react with lower species. One such factor is G-CSF, which recently has been purified [3], molecularly cloned [4] and expressed as a recombinant protein [5]. The cDNA of human G-CSF exhibits a 70% sequence homology to murine G-CSF [6] and in vivo has demonstrated biological activity in mice [7, 8]. G-CSF administration has been shown to significantly increase total granulocyte numbers in normal animals [9, 10] and in drug-induced myelosuppressed animals [8, 11-13], to enhance resistance to microbial infections in neutropenic cyclophosphamide-treated animals [14] and to increase survival after lethal doses of cyclophosphamide [15]. Furthermore, G-CSF has been shown to produce a variety of effects on mature granulocytes, including prolongation of survival in vitro [16], augmentation of antibody-dependent cytotoxicity [17, 18], and enhancement of phagocytosis [1]. Recent clinical trials with cancer patients undergoing cytotoxic chemotherapy have also revealed granulocytopoietic effects of G-CSF in humans [19-21].

Neutropenia is a major factor contributing to infection-induced morbidity and mortality after radiation exposure. Agents capable of enhancing host resistance to infection and/or the regeneration of hemopoietic elements necessary for efficient host defense mechanisms could be useful in circumstances of myelosuppression resulting not only from radiotherapy, but also from accidental radiation exposures, such as those occurring recently in Chernobyl (USSR), Goiania (Brazil) and El Salvador (San Salvador). *Kobayashi et al.* demonstrated that daily administration of G-CSF to mice exposed to 3 Gy or 5 Gy of total-body radiation accelerated peripheral blood leukocyte recovery by five to ten days and stimulated femoral and splenic granulocyte-macrophage colony-forming cells (CFC-gm) regeneration [22]. The studies presented in this paper expand on *Kobayashi's* original work and describe the ability of therapeutically administered G-CSF to increase survival and to stimulate hemopoietic regeneration in more severely irradiated mice.

Materials and Methods

Mice

C3H/HeN female mice (~ 20 g) were purchased from Charles River Laboratories (Raleigh, NC). Mice were maintained in an accredited American Association for Accreditation of Laboratory Animal Care facility in micro-isolator cages on hardwood-chip, contact bedding and were provided commercial rodent chow and acidified water (pH 2.5) ad libitum. Animal rooms were equipped with full-spectrum light from 6 a.m. to 6 p.m. and were maintained at $70 \pm 2°F$ with $50 \pm 10\%$ relative humidity using at least 10 air changes per hour of 100% conditioned fresh air. Upon arrival, all mice were tested for *Pseudomonas* and quarantined until test results were obtained. Only healthy mice were released for experimentation. All animal experiments were approved by the Institute Animal Care and Use Committee prior to performance.

Recombinant Human G-CSF (rhG-CSF)

rhG-CSF was provided by Amgen (Thousand Oaks, CA). This rhG-CSF (Lot #600) was derived from *E. coli* and had a specific activity of 1×10^8 U/mg as assayed by the

CFC-gm assay using normal human bone marrow cells. Endotoxin was undetectable based on the *Limulus* amebocyte lysate assay. rhG-CSF was administered s.c. at a dose of 2.5 μg/day.

Irradiation

The cobalt-60 source at the Armed Forces Radiobiology Research Institute was used to administer bilateral total-body gamma radiation. Mice were placed in ventilated plexiglass containers and irradiated at a dose of 0.4 Gy/min. Dosimetry was determined by ionization chambers. Hemopoietic recovery studies were performed after 6.5 Gy irradiations. Survival and endogenous spleen colony studies were performed after 8.0 Gy irradiations.

Survival Assays

Irradiated mice were returned to the animal facility and cared for routinely. Survival was checked and recorded daily for 30 days: on day 31, surviving mice were euthanized by cervical dislocation. Each treatment group consisted of 10 mice. Experiments were repeated 2-3 times. The percentage of mice surviving 30 days post-exposure was used to analyze survival data.

Granulocyte-Macrophage Colony-Forming Cell (CFC-gm) Assay

Hemopoietic progenitor cells committed to granulocyte and/or macrophage development were assayed using an agar CFC-gm assay. Mouse endotoxin serum (5% v/v) was added to feeder layers as a source of colony-stimulating activity. Colonies (> 50 cells) were counted after 10 days of incubation in a 37°C humidified environment containing 5% CO_2. Triplicate plates mere cultured for each cell suspension, and experiments were repeated 2-3 times. The cell suspensions used for each assay represented tissues from 3 normal, irradiated, or treated irradiated mice at each time point. Cells were flushed from femurs with 3 ml of McCoy's 5A medium (Flow Labs, McLean, VA) containing 10% heat-inactivated fetal bovine serum (Hyclone Labs, Logan, UT). Spleens were pressed through a stainless-steel mesh screen, and the cells were washed from the screen with 6 ml medium. The number of nucleated cells in the suspensions was determined by a Coulter counter. Femurs and spleens were removed from mice euthanized by cervical dislocation.

Peripheral Blood Cell Counts

Blood was obtained from cervically dislocated mice via cardiac puncture using a heparinized syringe attached to a 20-gauge needle. White blood cell (WBC), red blood cell (RBC) and platelet (PLT) couı ts were performed using a Coulter counter. In addition, blood smears were prepared and stained with Diff-Quik to perform WBC differential counts.

Endogenous Spleen Colony-Forming Unit (CFU-s) Assay

The endogenous CFU-s assay was used to measure hemopoietic stem cell recovery in irradiated mice. Mice were exposed to 9.0 Gy of radiation to partially ablate endogenous hemopoietic stem cells. Twelve days later, mice were euthanized by cervical dislocation and spleens were removed. The spleens were fixed in Bouin's solution, and grossly visible spleen colonies arising from the clonal proliferation of surviving endogenous hemopoietic stem cells were counted. Each treatment group consisted of 5 mice. Experiments were repeated 2-3 times.

Statistics

Results of replicate experiments were pooled and are represented as the mean $\pm$ SE of pooled data. Student's t test was used to determine statistical differences in all but survival data; survival data v ere analyzed using the generalized Savage (Mantel-Cox) procedure. Significance level was set at $p < 0.05$.

Table I. Effect of rhG-CSF on peripheral blood, bone marrow and splenic cellularity in non-irradiated C3H/HeN mice

	Saline[a]	rhG-CSF[b]						
		Day after initiation of rhG-CSF						
		1	4	7	9	10	14	17
WBC/ml	5.7	6.0	7.9[c]	9.4[c]	9.4[c]	6.7[c]	5.2	6.0
($\times 10^6$)	±	±	±	±	±	±	+	±
	0.2	0.8	0.2	0.5	0.3	0.3	0.2	0.5
PMN/ml	1.7	2.5[c]	3.7[c]	4.2[c]	3.5[c]	2.6[c]	1.4	1.7
($\times 10^6$)	±	±	±	±	±	±	±	±
	.04	0.1	0.2	0.2	0.2	0.2	0.1	0.1
RBC/ml	6.5	6.2	6.1	6.9	6.3	6.8	6.4	6.4
($\times 10^9$)	±	±	±	±	±	±	±	±
	0.2	0.2	0.3	0.5	0.2	0.3	0.2	0.5
Cells/femur	4.9	3.5[c]	3.7[c]	4.1	5.6[c]	6.6[c]	6.1[c]	4.5
($\times 10^6$)	±	±	±	±	±	±	±	±
	0.2	0.2	0.2	0.6	0.2	0.3	0.3	0.2
Cells/spleen	1.1	0.7[c]	1.6[c]	1.7[c]	1.7[c]	1.3[c]	1.0	1.6[c]
($\times 10^8$)	±	±	±	±	±	±	±	±
	.05	.08	.02	.05	.08	.06	.10	.09

[a] Average values obtained from non-irradiated mice treated with saline on days 0-9 and assayed on days 1, 4, 7, 9, 10, 14 and 17. No statistical differences were observed in data obtained on individual days; therefore, all saline data were pooled.

[b] rhG-CSF (2.5 μg/mouse/day) was administered s.c. on days 0-9.

[c] $p < 0.05$, with respect to saline values

Results

Hemopoietic Activity of rhG-CSF in Normal C3H/HeN Mice

Because the G-CSF proposed for use in these studies was a recombinant human preparation, preliminary studies were performed to be certain that this rhG-CSF would function as a hemopoietic stimulant in our mice. In these studies, non-irradiated C3H/HeN mice were administered rhG-CSF for 10 days, and at various times after the initiation of rhG-CSF administration, peripheral blood, femoral and splenic cellularity, and femoral and splenic CFC-gm content were evaluated. Peripheral WBC values increased to approximately 165% of normal values by day 7, maintained this level through day 9, then rapidly returned to normal after cessation of rhG-CSF administration (Table I). The increased WBC count observed after rhG-CSF treatment was due primarily to an increase in polymorphonuclear neutrophils (PMNs; Table I). In contrast to the effect of rhG-CSF administration on WBC values, RBC values remained relatively constant, fluctuating within approximately 5% of normal values throughout the experiment (Table I). One day after the initial rhG-CSF administration, femoral cellularity decreased

to 71% of normal values, then gradually increased to 135% by day 10 (1 day after cessation of rhG-CSF administration) and returned to normal values by day 17 (Table I). Femoral CFC-gm content followed a similar pattern, decreasing to 52% of normal values 1 day after initiation of rhG-CSF administration, rising to approximately 160% on days 9 and 10, then falling to 118% by day 17 (Fig. 1). In the spleen, a decrease to 64% of normal cellularity was seen on day 1 after initiation of rhG-CSF administration (Table I). This was followed by an increase to approximately 155% of normal values on days 7 and 9, a return to normal on day 14 and a second increase to 145% of normal values on day 17. Splenic CFC-gm content increased significantly during rhG-CSF administration, reaching a peak of 1335% of normal values on day 7, then rapidly declining to 459% by 1 day after cessation of rhG-CSF treatment (i.e., day 10) and returning to within normal values by day 17 (Fig. 2). These studies demonstrated that the rhG-CSF dose and injection protocol proposed for irradiation studies was capable of inducing significant hemopoietic stimulation in normal C3H/HeN mice.

Therapeutic rhG-CSF Administration Enhances Hemopoietic Recovery in Irradiated C3H/HeN Mice

To evaluate the ability of therapeutically administered rhG-CSF to enhance hemopoietic regeneration after radiation-induced myelosuppression, mice were exposed to a non-lethal dose of 6.5 Gy of cobalt-60 and treated with rhG-CSF on days 3-12 post-exposure. Initiation of rhG-CSF therapy was delayed 3 days post-exposure to allow some post-irradiation repair and regeneration of pluripotent and committed progenitor cells. On days 4, 7, 10, 12, 14, 17 and 20 post-irradiation, bone marrow and splenic cellularity and CFC-gm content were evaluated in non-irradiated, saline-treated mice (i.e., normal control mice) and in irradiated mice treated with either saline or rhG-CSF. Peripheral blood values were also evaluated in these animals on days 7, 12, 17 and 20 post-exposure.

Peripheral WBC values in both saline- and rhG-CSF-treated mice fell to approximately 20% of normal values by 7 days post-irradiation (Fig. 3A). WBC values recovered to only 60% of normal by day 20 in saline-treated mice, while WBC values reached 90% of normal values by day 20 in rhG-CSF-treated mice. An increase in the percentage of neutrophils was also observed in rhG-CSF-treated mice. Interestingly, accelerated recovery of both RBC and PLT values was also observed in rhG-CSF-treated mice (Figs. 3B and 3C). By day 20 post-exposure, RBC values in rhG-CSF-treated mice were 88% of normal values (compared to 55% in saline-treated mice) and PLT values were 66% of normal values (compared to 22% in saline-treated mice).

Based on cellularity and CFC-gm content, femoral and splenic hemopoietic regeneration were also enhanced significantly by rhG-CSF administration. Femoral cellularity in both saline- and rhG-CSF-treated mice decreased to approximately 20% of normal values by 4 days post-exposure (Table II). Interestingly, recovery of femoral cellularity in rhG-CSF-treated mice occurred more slowly than in saline-
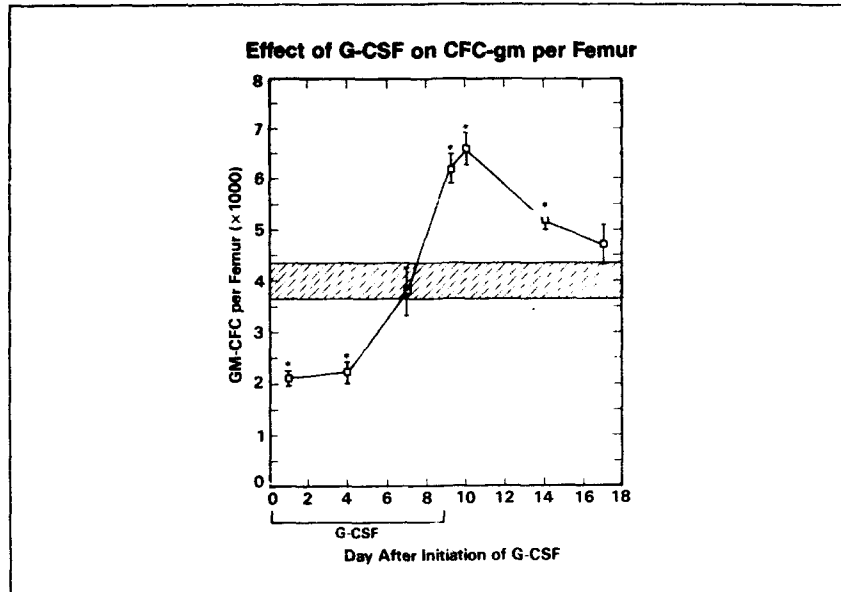
Effect of G-CSF on CFC-gm per Femur

Fig 1. CFC-gm per femur in non-irradiated C3H/HeN mice receiving 2.5 μg/day of rhG-CSF administered s.c. on days 0-9. Shaded area represents femoral CFC-gm content obtained in non-irradiated mice injected with saline. * = $p < 0.05$, with respect to saline controls.
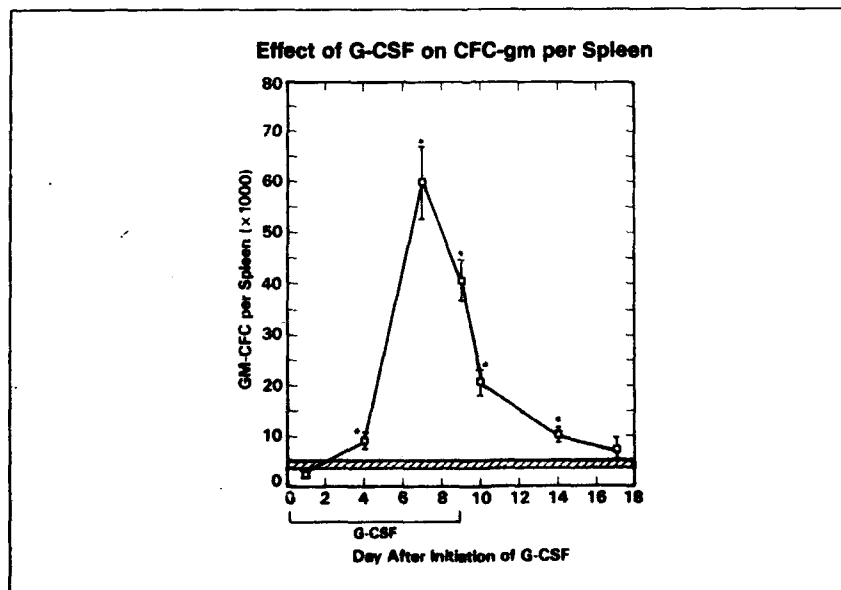
Effect of G-CSF on CFC-gm per Spleen

Fig. 2. CFC-gm per spleen in non-irradiated C3H/HeN mice receiving 2.5 μg/day of rhG-CSF administered s.c. on days 0-9. Shaded area represents splenic CFC-gm content obtained in non-irradiated mice injected with saline. * = $p < 0.05$, with respect to saline controls.
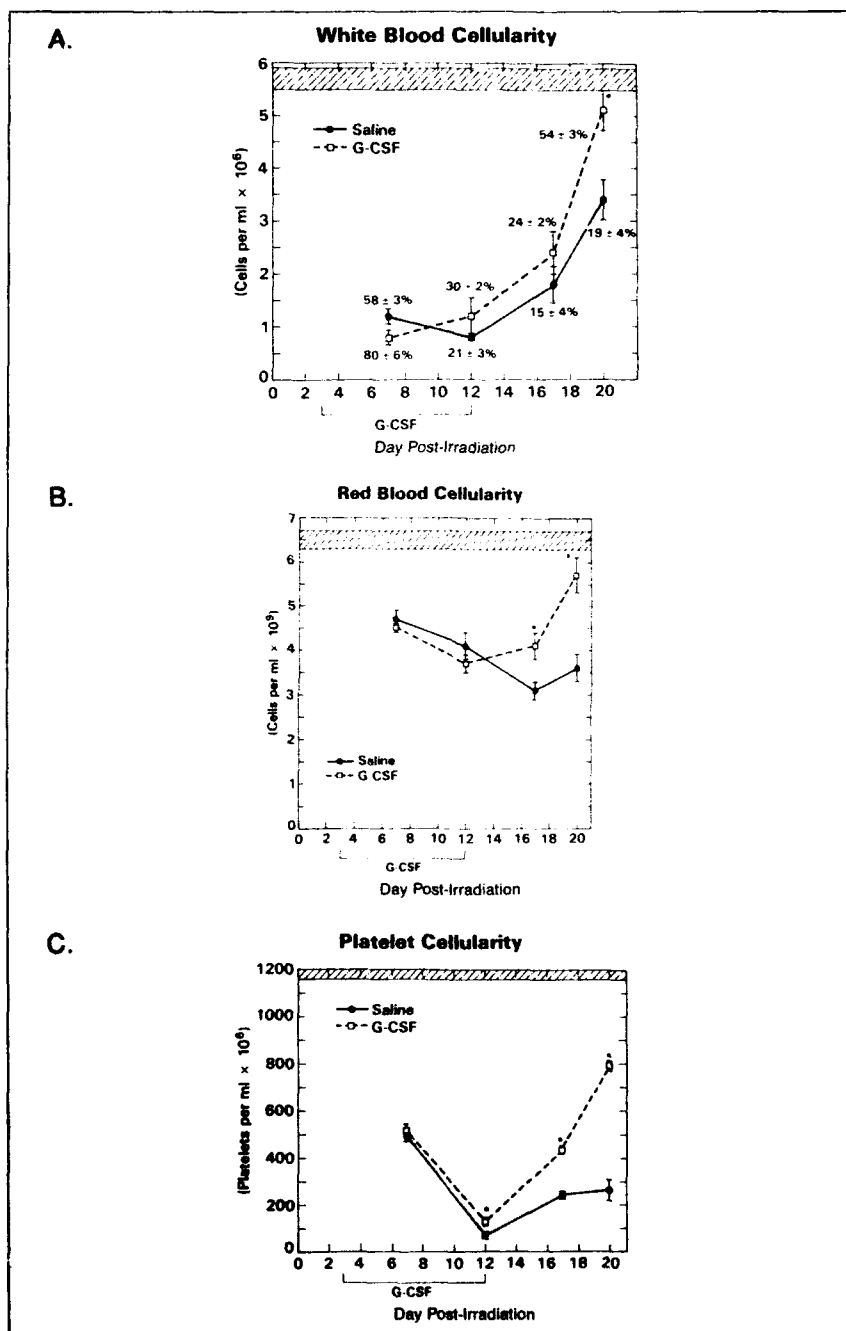
Fig. 3. Peripheral blood cellularity in C3H/HeN mice after 6.5 Gy. rhG-CSF (2.5 µg/day) or saline was administered s.c. on days 3-12. Shaded area represents values obtained in non-irradiated mice injected with saline. * = $p < 0.05$, with respect to irradiated saline-treated mice. A = WBC; values in parentheses indicate percentage of neutrophils at each time point. B = RBC. C = PLT.

Table II. Effect of rhG-CSF on bone marrow and splenic cellularity in irradiated C3H/HeN mice[a]

	Day post-irradiation						
	4	7	10	12	14	17	20
Cells/femur[b] (×10⁶)							
Saline	1.2 ± 0.1	2.1 ± 0.2	2.7 ± 0.2	2.6 ± 0.1	2.3 ± 0.2	1.8 ± 0.1	2.1 ± 0.1
rhG-CSF	0.8 ± 0.1	1.4[c] ± 0.2	2.1 ± 0.4	2.8 ± 0.2	2.6 ± 0.1	3.2[c] ± 0.1	3.8[c] ± 0.1
Cells/spleen[d] (×10⁸)							
Saline	.08 ± .006	.09 ± .003	.16 ± .01	.22 ± .005	.39 ± .03	.89 ± .06	1.60 ± .04
rhG-CSF	.10 ± .004	.11 ± .004	.27[c] ± .03	.38[c] ± .04	.47[c] ± .02	1.90[c] ± .07	1.70 ± .05

[a] rhG-CSF (2.5 µg/mouse/day) was administered s.c. on days 3-12 after 6.5 Gy radiation exposure.

[b] Average number of cells per femur in non-irradiated saline-treated mice was 4.9 ± 0.2 × 10⁶.

[c] $p < 0.05$, with respect to values obtained from irradiated and saline-treated mice

[d] Average number of cells per spleen in non-irradiated saline-treated mice was 1.1 ± 0.05 × 10⁸.

treated mice through day 10. However, enhanced cellular recovery was observed in rhG-CSF-treated mice on days 17 and 20. Femoral cellularity in rhG-CSF-treated mice was 78% of normal values by day 20, compared to 43% in saline-treated mice. Bone marrow CFC-gm recovery was also enhanced significantly in rhG-CSF-treated mice, increasing from 7% of normal values on day 12 post-exposure to 58% on day 20; CFC-gm recovery in saline-treated mice during this time increased from 2% to only 17% of normal values (Fig. 4). Splenic cellularity in both groups of mice decreased to approximately 10% of normal values until 10 days post-exposure (Table II). Recovery occurred in both treatment groups on days 10-20; it occurred more rapidly, however, in rhG-CSF-treated mice. By day 17 post-exposure, splenic cellularity in rhG-CSF-treated mice was 173% of normal values, compared to 81% in saline-treated mice. Recovery of splenic CFC-gm content in rhG-CSF-treated mice also increased dramatically compared to saline-treated mice (Fig. 5). By day 14 post-exposure, CFC-gm content in rhG-CSF mice had reached 63% of normal values, compared to 1% in saline-treated mice. In addition, CFC-gm contents in rhG-CSF mice were 937% and 505% of normal values on days 17 and 20, compared to 57% and 212% in saline-treated mice.
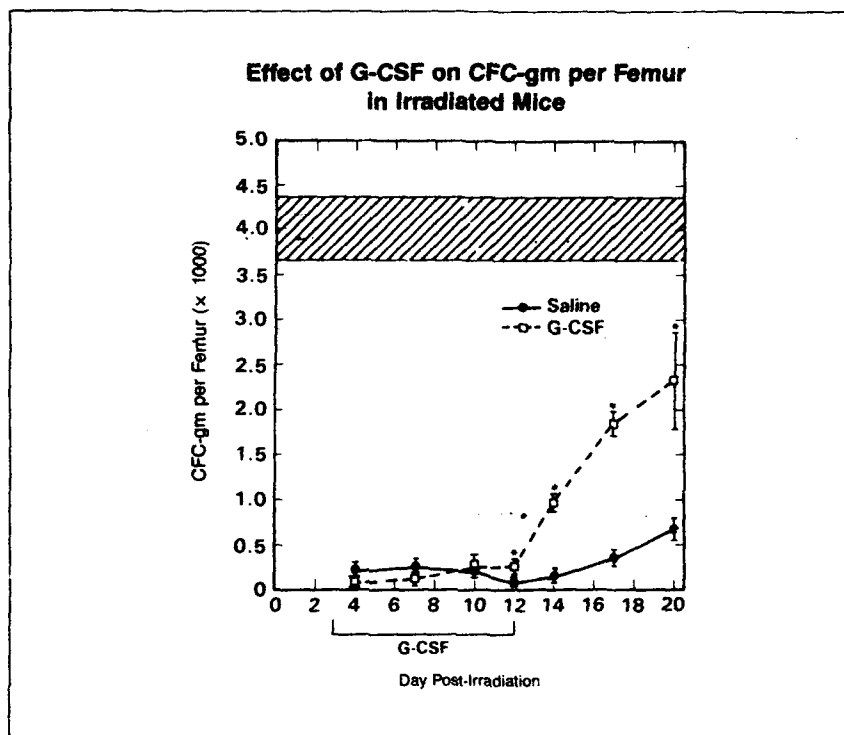
Fig. 4. CFC-gm per femur in C3H/HeN mice after 6.5 Gy. rhG-CSF (2.5 μg day) or saline was administered s.c. on days 3-12. Shaded area represents femoral CFC-gm content obtained in non-irradiated mice injected with saline. * = $p < 0.05$, with respect to irradiated saline-treated mice.

rhG-CSF therapy enhanced the recovery of not only WBC counts, but also RBC and PLT counts in irradiated mice. This finding suggested that rhG-CSF may stimulate hemopoiesis at a more primitive progenitor level than that of the CFC-gm. Because of this, the endogenous spleen colony assay was used to determine the effects of rhG-CSF therapy on multipotent hemopoietic stem cell recovery in irradiated mice. In these studies, mice were exposed to 8 Gy of radiation and injected with rhG-CSF on days 3-12, 1-12 or 0-12 post-irradiation. The first rhG-CSF treatment in the latter group was administered 1 h post-irradiation. The day 1-12 and day 0-12 injection treatments were added to these experiments to evaluate possible effects of rhG-CSF therapy on stem cell burnout. Mice treated with rhG-CSF on days 3-12 exhibited significantly more endogenous spleen colonies than did saline-treated mice (3.6 ± 0.4 vs. 1.3 ± 0.4), indicating that therapeutic rhG-CSF was capable of stimulating proliferation of multipotential hemopoietic progenitors (Fig. 6). Interestingly, when rhG-CSF therapy was initiated 1 day or 1 h post-irradiation, even greater CFU-s responses were observed.
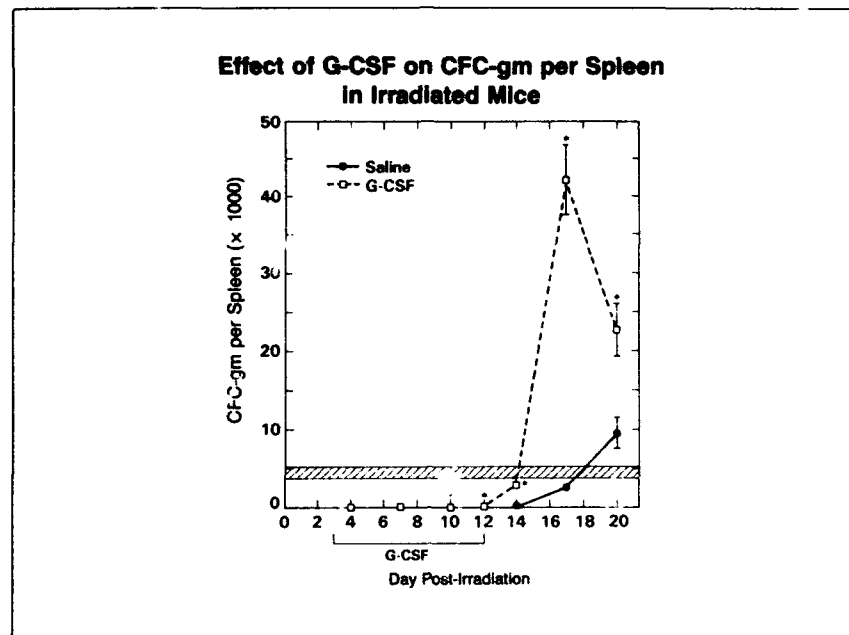
Effect of G-CSF on CFC-gm per Spleen in Irradiated Mice

Fig. 5. CFC-gm per spleen in C3H/HeN mice after 6.5 Gy. rhG-CSF (2.5 μg/day) or saline was administered s.c. on days 3-12. Shaded area represents splenic CFC-gm content obtained in non-irradiated mice inject d with saline. * = $p < 0.05$, with respect to irradiated saline-treated mice. Although difficult to detect. CFC-gm values in G-CSF-treated mice at days 10 and 12 (38.0 ± 9.0 and 49.0 ± 5.0, respectively) were significantly greater than CFC-gm values in saline-treated mice (0.4 ± 0.2 and 1.0 ± 0.2, respectively).

Therapeutic rhG-CSF Administration Enhances Survival of Irradiated C3H/ HeN Mice

To evaluate whether rhG-CSF-induced hemopoietic recovery could result in survival enhancement following severe radiation exposure, mice were exposed to 8 Gy of radiation and administered rhG-CSF on days 3-12, 1-12 or 0-12 post-irradiation. All rhG-CSF treatments enhanced survival (Fig. 7). Respectively, these treatments resulted in 57%, 70% and 95% survival, compared with 27% survival in saline-treated mice.

Discussion

Both radiation and chemotherapy destroy hemopoietic stem and progenitor cells, leading to a critical depletion of functional WBC within one to two weeks after treatment. As a result, the host is compromised with respect to natural defenses against exogenous infectious diseases, as well as endogenous gut-derived bacteria and their associated toxins [23-25]. Hemopoietic regeneration requires pluripotent stem cells capable of self-renewing, as well as differentiating into multi-
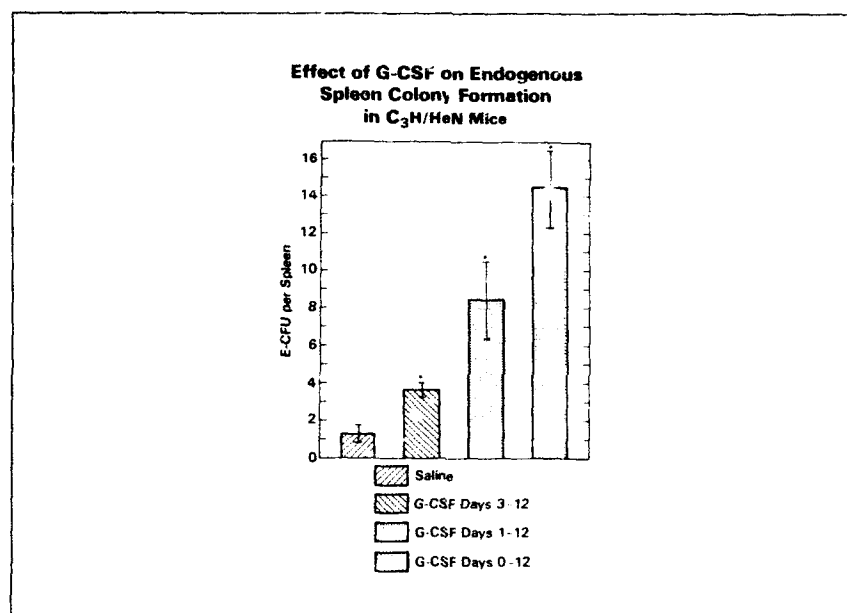
Fig. 6. Endogenous spleen colony formation in C3H/HeN mice after 8.0 Gy. rhG-CSF (2.5 μg/day) or saline was administered s.c. on days 3-12, 1-12 or 0-12 post-irradiation. * = $p < 0.05$, with respect to irradiated saline-treated mice.
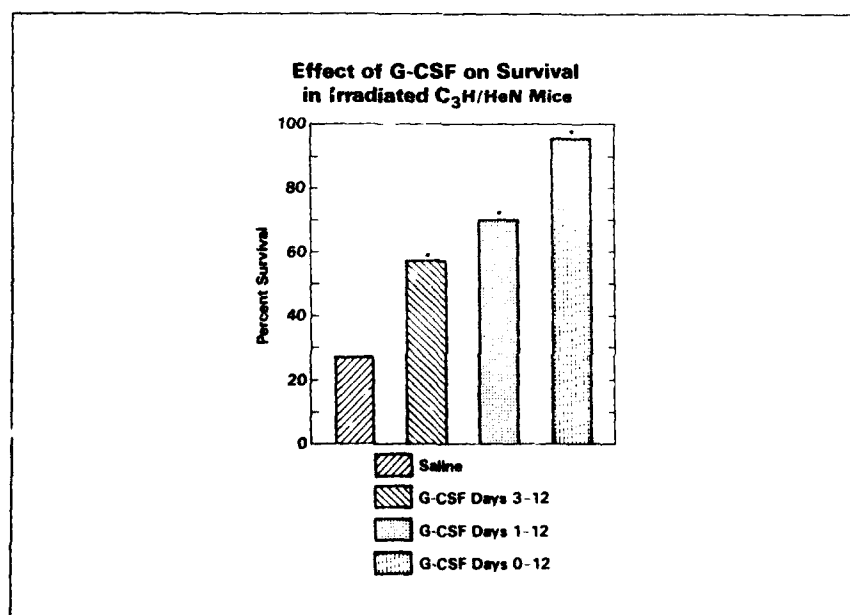

Fig. 7. Survival in C3H/HeN mice after 8.0 Gy. rhG-CSF (2.5 μg/day) or saline was administered s.c. on days 3-12, 1-12 or 0-12 post-irradiation (20-30 mice per group). * = $p < 0.05$, with respect to irradiated saline-treated mice.

potent and committed progenitors capable of giving rise to mature cells with specialized functions. These processes are regulated by specific molecules produced within specialized accessory cells constituting the hemopoietic microenvironment. Hence, in addition to direct effects on hemopoietic stem and progenitor cells, radiation and chemotherapy may also impair hemopoietic regeneration through destruction and/or injury to accessory cell populations necessary for the production of essential hemopoietic growth factors [26-29].

Until recently, with the exception of fluid, antibiotic and platelet support, no effective means to therapeutically treat myelosuppression and consequent sepsis and hemorrhage have been available [30-32]. However, developments in the isolation, purification and molecular cloning of hemopoietic growth factors have opened new possibilities regarding therapeutic enhancement of stem and progenitor cell recovery. rhG-CSF is one such recently cloned hemopoietic growth factor. Although originally recognized for its ability to stimulate the in vitro proliferation of progenitor cells giving rise to neutrophils [33], this factor has been demonstrated to also stimulate granulopoiesis in normal healthy animals in vivo. Our present studies in normal C3H/HeN mice reconfirmed such in vivo data obtained by others in normal C57BL/6 mice [7, 15], normal hamsters [10], normal canines [34], and normal primates [12] in which rhG-CSF administration was shown to increase peripheral WBC counts, as well as to increase CFC-gm progenitors. In addition to in vitro and in vivo effects on granulocyte proliferation and differentiation, rhG-CSF has also been demonstrated to prime and/or activate mature granulocytes to function more efficiently [1, 17, 18].

Several studies have demonstrated the ability of rhG-CSF therapy to enhance recovery from drug-induced myelosuppression. For example, hemopoietic regeneration of peripheral blood neutrophils in hamsters treated with 5-fluorouracil (5-FU) was accelerated two days with rhG-CSF therapy [10]. Similar peripheral effects as well as stimulatory effect on CFC-gm recovery have also been demonstrated in mice treated with 5-FU [8] or cyclophosphamide [14]. More recently, the ability of rhG-CSF therapy to accelerate granulocyte recovery in cyclophosphamide-treated primates [12] and humans receiving a variety of chemotherapeutic agents [4, 20, 21, 35] has also been demonstrated.

In the studies described in this paper, we additionally demonstrate the ability of rhG-CSF to enhance recovery from severe radiation-induced myelosuppression. Furthermore, survival in severely irradiated mice was increased by rhG-CSF treatment, suggesting that the mature cells (presumably the neutrophils) resulting from accelerated hemopoietic recovery were capable of enhancing host resistance to otherwise lethal post-irradiation opportunistic infections. This appears to be consistent with the fact that rhG-CSF has been demonstrated to increase survival [15] and to enhance resistance to microbial infection in cyclophosphamide-treated neutropenic mice [14]. Our current studies in mice confirm and expand our previous work in which enhanced peripheral blood neutrophil recovery, increased bone marrow hemopoiesis and increased survival were observed in irradiated canines

receiving more extended courses of rhG-CSF therapy [36]. These studies also confirm and expand previous work by *Kobayashi et al.* who demonstrated accelerated hemopoietic regeneration in rhG-CSF-treated mice exposed to less severe 3-5 Gy doses of total-body radiation [22].

In this study, rhG-CSF therapy accelerated hemopoietic recovery by four to five days based on femoral and splenic CFC-gm values. Because CFC-gm progenitors are ultimately derived from multipotent hemopoietic progenitor cells, we suspected that intensive rhG-CSF therapy may drain multipotent stem cell pools by driving these cells toward differentiation at the expense of self-renewal. The CFU-s data presented in these studies, however, suggests that this does not occur. In fact, rhG-CSF administration actually increased CFU-s numbers, with the greatest increase being observed when rhG-CSF therapy was initiated 1 h post-irradiation. The survival-enhancing effect of rhG-CSF therapy was also best when initiated 1 h post-irradiation.

Contrary to the in vitro effects of rhG-CSF, which appear to be specific for granulocyte proliferation, differentiation and function, in vivo rhG-CSF therapy stimulated regeneration of multiple hemopoietic cell lineages. Specifically, WBC, RBC and PLT recoveries were all accelerated in rhG-CSF-treated mice. This discrepancy between in vitro and in vivo rhG-CSF effects suggests that the multilineage in vivo effects of rhG-CSF may be indirectly mediated. For example, GM-CSF has been demonstrated to stimulate the release of interleukin 1 from mature granulocytes [27, 37], which in turn has been demonstrated to stimulate the release of other pleotrophic hemopoietic cytokines, such as interleukin 6, GM-CSF, G-CSF and M-CSF from endothelial cells and fibroblasts [38-41]. If rhG-CSF similarly affects granulocytes, rhG-CSF-mediated stimulation of multipotential progenitor cell proliferation could be postulated via such a cascade.

In conclusion, these studies have demonstrated the ability of therapeutically administered rhG-CSF to accelerate hemopoietic regeneration and to increase survival after radiation-induced myelosuppression. rhG-CSF therapy accelerated the regeneration of multiple hemopoietic lineages and, contrary to stem cell burn out, actually appeared to stimulate multipotent stem cell proliferation. rhG-CSF should offer great promise for the treatment of radiation and/or drug-induced myelosuppression and its infectious consequences.

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